

Letters to the Editor

Correspondence re: E. Farber, Cell Proliferation as a Major Risk Factor for Cancer: A Concept of Doubtful Validity. *Cancer Res.*, 55: 3759-3762, 1995.

Letter

The September 1, 1995 issue of *Cancer Research* features a "Perspectives in Cancer Research" article on cell proliferation as a risk factor for cancer (1). Emmanuel Farber offers the provocative concept that cell turnover and replication have little or no impact upon the risk of cancer induction. His proposal, however, includes conceptual errors that must be corrected:

1) "It is now well documented that atrophic gastritis, with low levels of cell proliferation, not hyperplastic or hypertrophied gastritis, is a risk factor for the development of cancer."

On the contrary, the rate of cell turnover with autoimmune atrophic gastritis is actually increased in response to accelerated cell loss in the glandular epithelium (2). Intestinal metaplasia of the atrophic stomach is also associated with increased risk of cancer, as is Barrett's metaplasia of the distal esophagus, and each of these is characterized by increased rates of cell replication (3, 4). A similar phenomenon is observed with ulcerative colitis (5) and celiac disease (6). These two conditions, like autoimmune gastritis, are associated with an increased risk of cancer induction.

2) "It is now well documented that many, if not all, genotoxic carcinogens are inhibitors of DNA synthesis and/or cell proliferation."

The described phenomenon is an effect of the induction process rather than a risk factor for induction.

3) "Pregnancy is associated with a vigorous cell proliferation of all epithelial cells of the breast, yet is associated with decreased risk for breast cancer."

Women are especially vulnerable to the effects of X-ray-induced genotoxic damage during their adolescent years, when glandular proliferation is at its peak. This accounts for the increased risk of breast cancer among women who have received X-ray therapy for Hodgkin's disease during adolescence (7) and the limitation of increased breast cancer risk among Hiroshima women exposed to the atomic bomb to those who were adolescents or young adults at the time of exposure (8).

Increased vulnerability to cancer induction need not result in cancer if the vulnerable cells are not challenged by a genotoxic event. This probably accounts for the infrequent cancers in the human jejunum and ileum. The bacterial counts of the small bowel are much lower than those of the colorectum, and anaerobes are usually absent (9). *Clostridia*, *Bacteroides* sp., and coliform organisms are usually absent in the proximal small bowel or are present in only very small numbers. It has been argued that carcinogens in the colon are generated from bile salts as metabolites of anaerobic bacteria (10), and cancer vulnerability is not tested in their absence. This also accounts for the late onset of some cancers in families affected by hereditary nonpolyposis colon cancer. This dominant mutation in mismatch repair genes has low penetrance (11). The defect does not surface in the absence of genotoxicity.

Although the risk of cancer induction of some cancers may be unrelated to the rate of cell proliferation, it would seem premature to discard cell turnover as a risk factor in all primary sites. It would seem to have an especially strong influence upon the risk of cancer of the gastrointestinal tract.

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Letter

The article in *Cancer Research* by Emmanuel Farber (1), which attacks the idea that cell division is important in cancer causation, did not deal with the literature adequately or critically. Mutagenesis, and thus carcinogenesis, is increased by increasing either DNA damage or cell division in cells that are not discarded. There is, in fact, quite persuasive evidence that cell division is an important factor in mutagenesis and carcinogenesis.

1) There is enormous endogenous DNA damage from normal oxidation, and the evidence suggests that oxidative damage is a major factor not only in aging but in the degenerative diseases of aging such as cancer (2). The steady-state level of oxidative damage in DNA is over one million oxidative lesions per rat cell (2). Thus, because there is endogenous DNA damage, the cell division rate must be a factor in converting lesions to mutations and thus cancer (3). Raising the level of either DNA lesions or cell division will increase the probability of cancer. Just as DNA repair protects against lesions, p53 guards the cell cycle and protects against cell division if the lesion level gets too high; however, neither defense is perfect (4). Cell division is also a major factor in loss of heterozygosity through nondisjunction and other mechanisms (3).

2) Chronic cell division is plausible as the major reason that more than half the chemicals are classified as carcinogens when tested at the MTD¹ in standard rodent cancer bioassays (3, 5, 6). Of the chemicals tested in both rats and mice, 60% are positive; even among the known nonmutagens, 49% are carcinogenic (among the mutagens, 78% are carcinogenic; Ref. 5). The high positivity rate is consistent for synthetic chemicals, natural chemicals (99.9% of the chemicals humans are exposed to are natural; Refs. 5 and 6), natural pesticides, and chemicals in roasted coffee, and the proportion that is positive has not changed through the years of testing (5). Half the drugs in the *Physicians' Desk Reference* for which animal cancer test results are reported are carcinogenic (7). The Innes series of tests from 1969 of 119 synthetic chemicals, consisting mainly of all of the commonly used pesticides of the time, is frequently cited as evidence that the proportion of carcinogens in the world of chemicals is low because only 9% were judged positive. We have pointed out that these tests were only in mice and were quite deficient in power compared to modern tests. We have now reanalyzed Innes by asking whether any of the Innes-negative chemicals have been retested using current protocols. We found that 34 have been retested, and 16 were carcinogenic, again about half.

What is the explanation for the high positivity rate in high-dose animal cancer tests? We have rejected bias in picking more suspicious chemicals as the major explanation for the results for numerous reasons (6). One explanation for a high positivity rate that is supported by an ever-increasing array of papers is that the MTD of a chemical can cause chronic cell killing and cell replacement in the target tissue, a risk factor for cancer that can be limited to the high dose. Thus it seems likely that a high proportion of the chemicals in the world may be "carcinogens" if run through the standard rodent bioassay at the MTD, but this will be primarily due to the effects of high doses for the nonmutagens and to a synergistic effect between cell division at high doses and DNA damage for the mutagens (3, 4). *Ad libitum* feeding in the standard bioassay can also contribute to the high positivity rate (8), plausibly by increasing cell division due to high caloric intake (3, 8).

3) Many studies on rodent carcinogenicity show a correlation between cell division at the MTD and cancer [reviewed in Refs. 3 and 9 and by Cohen and Ellwein in the accompanying letter (10)]. Cunningham *et al.*, in 9 papers, have analyzed 15 chemicals at the MTD, 8 mutagens and 7 nonmutagens, including several pairs of mutagenic isomers, one of which is a carcinogen and one of which is not (11, 12). A perfect correlation was observed; the nine chemicals causing cancer caused cell division in the target tissue, and the six chemicals not causing cancer did not. A similar result has been found in the analyses of Mirsalis (13), *e.g.*, both dimethyl nitrosamine and methyl methane sulfonate methylate liver DNA and cause unscheduled DNA synthesis, but dimethyl nitrosamine causes both cell division and liver tumors, whereas methyl methane sulfonate does neither. A recent study on the mutagenic dose response of the carcinogen ethylnitrosourea concludes that cell division is a key factor in its mutagenesis and carcinogenesis (14). At high doses, chloroform induces liver cancer and sodium saccharin induces bladder cancer by chronic cell division (see accompanying letters; Refs. 10 and 15).

4) The large body of epidemiological literature reviewed by Preston-Martin, Henderson, and colleagues (16, 17) shows that increased cell division by hormones and other agents can increase human cancer.

5) The evidence marshalled against the idea is not persuasive: the critical factor is chronic cell division in stem cells, not in cells that are discarded. The papers that Dr. Farber quotes as arguing against a role

of cell division in particular cases of carcinogenesis either did not measure cell division, did not show that it was chronic, or did not differentiate between cells being discarded and stem cells.

Dr. Farber's summary in the sentence, "This new emphasis in cancer prevention, as nonexplicitly implied from the writings of Ames *et al.*, would encourage us to pay much less attention to our environment, such as smoking and occupation, than we do currently," seems both illogical and unfair, and is a misreading of our papers. We have been very explicit that cessation of smoking, improving diet, and controlling infections and chronic inflammation are the important ways to lower cancer rates, as well as occupational regulation for the small amount of cancer due to occupational factors (4). Biomedical research will lead to many future prevention strategies. What we do think is that taking cell division into account will make priority setting in cancer prevention more effective. Regulatory policy aimed at reducing tiny exposures to synthetic rodent carcinogens while ignoring the enormous natural background (6) has confused the public about what factors are important for preventing cancer (4) and has diverted resources from more important risks (4, 18); the costs dwarf the expenditures for biomedical research.

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¹ The abbreviation used is: MTD, maximum tolerated dose.

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Letter

Emmanuel Farber, in his "Perspectives in Cancer Research" article (1), raises questions concerning the role of cell proliferation as a major risk factor for cancer. This is somewhat puzzling since he states in his article that "it is axiomatic that cell proliferation plays an important and even critical role in many steps in cancer development." For genotoxic substances, he even states that cell proliferation is actually required. However, he then proceeds to cite several examples where there is an apparent discrepancy between the presence or absence of cell proliferation and cancer risk.

Some of the discrepancies described by Dr. Farber arise from a misinterpretation of the key features underlying the influence of cell proliferation on carcinogenesis. Dr. Farber focuses on mitotic rates (or labeling index), whereas in fact the key issue is the number of cell divisions that actually occur (2, 3). Thus, if the size of the cell pool increases, which happens after administration of some peroxisome proliferators such as clofibrate or diethylhexylphthalate, the total number of cell divisions is actually increased, although the cell division rate may be elevated above that of controls only for a short period of time, if at all. Similarly, phenobarbital does not seem to significantly increase cell mitotic rates, especially in normal hepatocytes. Instead, it seems to inhibit apoptosis, especially in liver nodules. This results in an accumulation of cells in these nodules, and although the rate of cell division may remain constant, the total number of cell divisions in these nodules is significantly increased for a prolonged period of time. Cell proliferation can take the form of an increase in the mitotic rate of cells or an increase in the size of the cell population, or both.

Further confusion arises due to a lack of consideration that the dividing cells must be in the pluripotential cell population of the tissue, *i.e.*, the cells that have the potential to give rise to tumors (2, 4, 5). For example, certain liver mitogens, such as lead nitrate, greatly increase cell proliferation in rat hepatocytes, but this is largely in cells that are tetraploid or greater ploidy. Cell division among diploid hepatocytes does not seem to be increased. The diploid cells are the ones that give rise to both the hyperplastic nodules and, ultimately, the liver tumors. Another example deals with cell proliferation within the colon. Adenomatous polyps of the colon are preneoplastic, whereas hyperplastic polyps are not. Although both represent increased cell proliferation, the former are a proliferation of the crypt cells of the colon, the presumed stem cells, whereas the hyperplastic polyps involve proliferation of differentiated cells. Dr. Farber (1) mentions the example of psoriasis. Again, this is predominantly a proliferation of differentiating keratinocytes rather than a proliferation of the stem cell population of the skin. The preventive effect of pregnancy on breast cancer also demonstrates the influence of having differentiated

cells account for an increasing proportion of the total cell population within the at-risk tissue. Although early in pregnancy there is a marked proliferative effect in the breast, later in pregnancy the critical cells differentiate, removing them from the pool of cells that can evolve into tumors (6).

The basis for cell proliferation being important in carcinogenesis, and therefore in risk assessment, is the realization that DNA replication does not have 100% fidelity. The mechanisms responsible for mistakes during DNA replication are being identified and include oxidative processes, deamination, formation of exocyclic adducts, and depurination. Replication mistakes occur by normal endogenous processes, most, but not all of which are repaired. If the unrepaired mistake happens to occur in a gene necessary for the development of cancer, cancer risk is increased. Unless one assumes that DNA replication has 100% fidelity, increased cell replication within the susceptible pluripotential cell population of the tissue must be considered a risk factor for carcinogenesis because it increases the opportunities for critical genetic mistakes to occur.

This biological model of carcinogenesis relies heavily on quantitative aspects of cell kinetics in representing the apparent stochastic nature of cancer development. This was described by Knudson (7) in his classic paper regarding the development of sporadic or hereditary retinoblastoma, in which somatic cell DNA replication is the source of the two genetic mistakes that occur in sporadic retinoblastoma development and the second genetic mistake in hereditary retinoblastoma. Exposure to an exogenous carcinogenic agent is not required. Retinoblastomas also illustrate the necessity of cell division in the stem cell population for cancer to occur. Once retinoblasts (like other neuroblasts) stop dividing in later childhood, retinoblastomas no longer develop.

Nonlinear relationships between cellular parameters and cancer risk lead to empirical results that can be intuitively difficult to grasp or interpret using more simplified views of carcinogenesis, as was the case with the ED₀₁ study (2, 5, 8). In that study, liver tumors were induced by 2-acetylaminofluorene at all doses (30–150 ppm), and the incidence increased approximately linearly with respect to dose. However, bladder tumors occurred only at doses of 60 ppm and above. DNA adducts were formed in both tissues at a level linear with respect to dose (5–150 ppm). Increased cell proliferation was not observed in the liver across the entire dose range of the study, but it was increased in the bladder at doses of 60–150 ppm, the same dose range within which tumor incidence was increased (8).

Dr. Farber (1) mentions the very high mitotic rate of the normal small intestine. Investigations as to why this does not translate into a comparatively high risk of cancer should give us significant insight into the relationship of cell proliferation and carcinogenesis. It is likely to be related to the unusual pattern of differentiation in the small intestine, but this is not yet proved. Interestingly, small intestinal adenocarcinomas seem to occur only after metaplastic transformation to a colonic type of epithelium with the stem cells at the base of the crypts. Just as intense investigation of what seemed to be discrepancies between DNA adduct formation and cancer risk led to further insights into our understanding of carcinogenesis, investigation into seeming discrepancies between cell proliferation and carcinogenesis can also provide us with insights into the carcinogenic process.

There are far too many examples that reinforce the relationship between cell proliferation and cancer risk for this to be treated as questionable. The relationship is most readily visualized in evaluating the carcinogenicity of calculi in the lower urinary tract (2, 9). It makes no difference whether the calculus is formed secondary to administration of high doses of various chemicals, to alterations of physiological processes (such as increased uric acid excretion), or to surgical implantation of a pellet, there is an increased risk of bladder tumor

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There are far too many examples that reinforce the relationship between cell proliferation and cancer risk for this to be treated as questionable. The relationship is most readily visualized in evaluating the carcinogenicity of calculi in the lower urinary tract (2, 9). It makes no difference whether the calculus is formed secondary to administration of high doses of various chemicals, to alterations of physiological processes (such as increased uric acid excretion), or to surgical implantation of a pellet, there is an increased risk of bladder tumor

development. There is variation between species in the susceptibility to this effect, with the rat apparently more susceptible than the mouse, and rodents in general more susceptible than humans. There is no direct interaction with DNA in these irritation situations, but there is the potential for enormous increases in the number of cell divisions, due to both increased cell number and increased cell mitotic rates. The implanted pellets are made with substances such as paraffin wax, cholesterol, and even glass or stainless steel. What carcinogenic mechanisms can even be postulated other than that based on the enormous increase in cell proliferation? If pellets are not present, or if the dose of the administered chemical is inadequate for urinary calculus formation, there is no increased cell proliferation and no increased risk of tumor development.

It is in the final paragraph of Dr. Farber's Perspectives article (1) in particular that he misinterprets data that have been published and their implications. He suggests that focusing on cell proliferation as a rate-limiting step in the carcinogenic process leads to a totally new and radical orientation to the primary prevention of cancer. Just the opposite is the case. As has been described by us (2-5), as well as others such as Henderson, Ames, Butterworth, Moolgavkar, and their colleagues (2), there are numerous examples in experimental animals and in human carcinogenesis studies in which exposure to environmental agents leads to increased proliferation and an increased risk of cancer. If anything, focusing on the relationship between cell proliferation and carcinogenesis provides a biological framework upon which to design studies to identify cancer-causing agents and delineate ways in which we might be able to prevent or slow cancer development. It also provides a more rational, biological basis for extrapolating risk as assessed in high-dose rodent studies to low-dose human exposures. In many instances, it is the combination of a genotoxic effect with increased cell proliferation that leads to a significant increase in cancer risk and its recognition in human populations (2, 4). This is certainly the case with cigarette smoke as it relates to both lung and bladder cancer (8). Most recently, this has been illustrated in the synergistic relationship between aflatoxin (genotoxic effect) and the hepatitis B virus (proliferative effect) in the etiology of hepatomas in certain parts of the world (10). Simultaneous exposure to agents that are genotoxic and other agents that cause increased proliferation produces a tremendous synergy with respect to cancer development. In contrast to what Dr. Farber (1) states, a focus on cell proliferation as a critical aspect in carcinogenesis helps bring better-informed attention on environmental agents that truly can increase cancer in humans.

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Letter

In his thought-provoking commentary, Emmanuel Farber (1) questions whether induced cell proliferation, *per se*, is a risk factor for the long process of cancer development. This point is already generally appreciated, and new proposals for improving dose selection and evaluation of cancer studies recommend that cell proliferation not be considered in isolation but rather in conjunction with additional information such as whether the chemical is DNA-reactive and whether the response represents direct mitogenic activity or is regenerative growth in response to accompanying necrosis and inflammation (2). Dr. Farber further implies that consideration of cell proliferation data would be counterproductive in prioritizing the relative risks of environmental carcinogens. In fact, cell proliferation and associated growth control issues are an integral part of almost every step in the carcinogenic process. Rather than being counterproductive, appropriate consideration of the pattern of induced cell proliferation can be critical in selecting appropriate doses for cancer studies, formulating more appropriate risk assessments, and prioritizing concerns for experimental carcinogens.

In a 1978 article entitled "Initiation of chemical carcinogenesis requires cell proliferation," Dr. Farber described a liver initiation-promotion model to study chemically induced cancer (3). In this study, he demonstrated that cell proliferation induced by a partial hepatectomy greatly enhanced the initiating potency of the mutagen being used by converting DNA adducts to true mutations before DNA repair could occur. Further, he used the liver mitogen α -hexachlorocyclohexane to provide a selective growth advantage for precancerous cells in the promotion phase of the protocol. A large body of literature substantiates that mitogenic stimulation of growth and events associated with cytotoxicity such as inflammation, nuclease release, and stimulation of regenerative cell proliferation can play critical roles in the process of chemical carcinogenesis (4). These articles document the following principles:

- 1) Every round of DNA replication involves the possibility of a low level of spontaneous mutation formation.
- 2) Cell replication enhances the effectiveness of endogenous and exogenous mutagens by converting DNA adducts to mutations before repair can occur. For example, initiation-promotion protocols employ both a mutagen and induced cell proliferation to produce mutations as the initiation portion of the procedure.
- 3) Organ-specific cell killing and necrosis can result in inflammation and release of nucleases, both of which can damage DNA.
- 4) Chronic treatment with a cytotoxic chemical can result in a

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development. There is variation between species in the susceptibility to this effect, with the rat apparently more susceptible than the mouse, and rodents in general more susceptible than humans. There is no direct interaction with DNA in these irritation situations, but there is the potential for enormous increases in the number of cell divisions, due to both increased cell number and increased cell mitotic rates. The implanted pellets are made with substances such as paraffin wax, cholesterol, and even glass or stainless steel. What carcinogenic mechanisms can even be postulated other than that based on the enormous increase in cell proliferation? If pellets are not present, or if the dose of the administered chemical is inadequate for urinary calculus formation, there is no increased cell proliferation and no increased risk of tumor development.

It is in the final paragraph of Dr. Farber's Perspectives article (1) in particular that he misinterprets data that have been published and their implications. He suggests that focusing on cell proliferation as a rate-limiting step in the carcinogenic process leads to a totally new and radical orientation to the primary prevention of cancer. Just the opposite is the case. As has been described by us (2-5), as well as others such as Henderson, Ames, Butterworth, Moolgavkar, and their colleagues (2), there are numerous examples in experimental animals and in human carcinogenesis studies in which exposure to environmental agents leads to increased proliferation and an increased risk of cancer. If anything, focusing on the relationship between cell proliferation and carcinogenesis provides a biological framework upon which to design studies to identify cancer-causing agents and delineate ways in which we might be able to prevent or slow cancer development. It also provides a more rational, biological basis for extrapolating risk as assessed in high-dose rodent studies to low-dose human exposures. In many instances, it is the combination of a genotoxic effect with increased cell proliferation that leads to a significant increase in cancer risk and its recognition in human populations (2, 4). This is certainly the case with cigarette smoke as it relates to both lung and bladder cancer (8). Most recently, this has been illustrated in the synergistic relationship between aflatoxin (genotoxic effect) and the hepatitis B virus (proliferative effect) in the etiology of hepatomas in certain parts of the world (10). Simultaneous exposure to agents that are genotoxic and other agents that cause increased proliferation produces a tremendous synergy with respect to cancer development. In contrast to what Dr. Farber (1) states, a focus on cell proliferation as a critical aspect in carcinogenesis helps bring better-informed attention on environmental agents that truly can increase cancer in humans.

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continual state of induced cell death and regenerative growth. This regenerative cell proliferation can increase the effectiveness of the production of DNA damage that is secondary to necrosis.

5) Measurement of induced regenerative cell proliferation is a valuable marker for cytolethality and necrosis and greatly facilitates measurement of the shape of the dose-response curve for these important end points. This information is a critical factor in selecting doses for cancer studies.

6) Many growth control genes, some of which are oncogenes or tumor suppressor genes, are transcriptionally induced during regenerative cell proliferation. Induced expression targets these genes because the DNA is unwound, making them more susceptible to endogenous or exogenous mutation induction.

7) Release of those growth factors responsible for stimulating regrowth of a damaged target organ may provide a selective growth advantage to precancerous cells.

8) In tissues where background prevalence of cancer is high, cell proliferation may potentiate endogenous processes to increase the incidence of cancer.

9) Because the above activities are secondary to cytolethality, the subset of carcinogens that act through a nongenotoxic-cytotoxic mode of action would not be expected to increase the cancer risk at nontoxic doses.

Initiation, promotion, and progression in the cancer process are highly dependent on the dose rate and total dose delivered to the target tissue and the particular experimental model and species under study. Thus, in understanding mechanisms, merely labeling a chemical as an "initiator" or "promoter" is inadequate. It is more instructive to consider each target site on a case-by-case basis to determine the manner in which mutations were induced or the way in which clonal growth of preneoplastic cells was affected (2). For example, non-DNA-reactive chemicals are perfectly capable of inducing cancer if given at high doses that produce chronic cell killing and regenerative cell proliferation. Examples of such nongenotoxic-cytotoxic carcinogens and their activities are saccharin-induced hyperplasia and subsequent tumors in the rat bladder (5), chloroform-induced necrosis and regenerative cell proliferation and subsequent tumors in the mouse liver (6), and terephthalic acid formation of urinary calculi and foreign body hyperplasia and subsequent bladder tumors (7). For such chemicals, it is crucial that their mode of action be considered in estimating potential human cancer risks at low levels of exposure.

Dr. Farber states, "This new emphasis in cancer prevention, as nonexplicitly implied from the writing of Ames *et al.*, would encourage us to pay much less attention to our environment, such as smoking and occupation, than we do currently." The opposite is true. Consider the following example of smoking *versus* a nongenotoxic carcinogen in the context of public education as part of cancer prevention efforts. Saccharin is a nongenotoxic-cytotoxic carcinogen dependent on extremely high doses to produce cancer in experimental animals with no indication of increased cancer risk in people under normal usage (5). In contrast, cigarette smoke is mutagenic and cytotoxic, and cancer associated with its use has claimed millions of lives. Yet, to the general public, the cancer warning on a cigarette package is practically the same as the cancer warning on a saccharin packet. By not considering mechanisms in prioritizing risks we fail to distinguish trivial from serious hazards, resulting in ineffective public education policies.

In a world with an ever-increasing population demanding more products, there are few simplistic solutions to environmental concerns such as banning the use of a particular chemical. Rather, we are usually faced with the choice of replacing one technology and corresponding list of toxicological hazards with another. The economic

impact of decisions that hinge on cancer risk assessments is immense, and those dealing with real-world issues need guidance from the cancer research community on setting priorities for the large and growing list of chemicals identified as carcinogens in high-dose animal studies. The default extrapolation procedure most commonly used in cancer risk assessment is the LMS¹ model, which assumes that all carcinogens act by a directly mutagenic mechanism of action and that even vanishingly low levels of exposure are associated with some risk of cancer. For example, the United States Environmental Protection Agency currently uses the LMS model applied to mouse liver tumor incidence data from a chloroform gavage study in B6C3F₁ mice (8) to estimate a virtually safe dose for inhaled chloroform. The resulting value is an airborne exposure concentration of 0.000008 ppm (9). Currently, reaching control levels this low is not technically feasible, and efforts to achieve this goal would require hundreds of millions of dollars.

In contrast, new extrapolation models incorporating mechanistic information are less uncertain and can provide more realistic risk estimates. For chemicals such as chloroform that act through a nongenotoxic-cytotoxic mode of action and for which a close correlation between cancer and regenerative cell proliferation has been established (6), no increased cancer risk would be expected at nontoxic doses of the agent (2). A 90-day chloroform inhalation study has demonstrated no increased necrosis or cell proliferation in the livers of B6C3F₁ mice at airborne concentrations of 10 ppm or less of chloroform (10). Estimation of a virtually safe dose by applying a safety factor in the range of 100–1000 yields a risk estimate that is based on inhalation data, is more consistent with the mechanism of action of chloroform, and is just as health protective as LMS-based estimates. Consideration of this kind of mechanistic data will allow resources to be better spent elsewhere in the fight against cancer rather than in trying to reduce exposures to unrealistic and unnecessarily low levels.

Use of mechanistic data to appropriately increase or decrease estimated cancer risks is vital to the scientific community and those we serve. Induced cell proliferation is one critical piece of information to be evaluated in the design and interpretation of experimental cancer studies and should be considered thoughtfully with all other information available.

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¹ The abbreviation used is: LMS, linearized multistage.

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Reply

I wish to thank Grant Stemmermann, Amy Noffsinger, and Cecilia Fenoglio-Preiser (1); Bruce Ames and Lois Gold (2); Samuel Cohen and Leon Ellwein (3); and Byron Butterworth (4) for their interesting responses to my Perspectives article on cell proliferation (5).

In the Perspectives article, I was concerned with the scientific evidence in support of the hypothesis that cell proliferation is a major risk factor for many different cancers. I consider that the most important scientific evidence consistent with such an hypothesis is the demonstration that cell proliferation, by itself, is sufficient to lead to cancer. Less convincing but still relevant evidence could be the demonstration in humans or animals that cell proliferation is the rate-limiting step in the cancer-development system in which more than one important factor is operating. This second type of scientific evidence is very difficult to come by because the rates of different steps in any system of cancer development are virtually impossible to measure, except under very controlled and synchronous experimental systems. Two such model systems are mentioned in paragraph #23 (5).

As discussed briefly in paragraphs #2, 5, 6, and 15 and in Table 1 (5), cell proliferation plays key roles at several steps in cancer development and cancer progression. Only someone totally unfamiliar with cancer, either clinical or experimental, would consider otherwise. However, the importance of cell proliferation does not *per se* make it a *major risk factor*.

The illustrative examples in the letter by Dr. Stemmermann *et al.* (1) strengthen this evaluation. They refer to the observations in the small and large intestine. Their point, of course, adds strength to my first contention that there seems *not* to be a system in which cell proliferation, active and vigorous, does by itself lead to cancer. The possible explanation or explanations offered simply attempt to pinpoint the possible nature of the other factors essential for cancer development in the intestine. The other examples in the colon by no means point to cell proliferation as the key component in the cancer development. Every chronic disease of the colon has so many other factors of possible importance, such as cell injury, inflammation, change in bacterial and other flora, etc., that it is not justified to single out, arbitrarily, cell proliferation.

Also, despite such discussions as referred to in their Ref. 2, the levels of cell proliferation in the colon are normally so vigorous that it is indeed doubtful that there is a requirement for even more vigorous cell proliferation for cancer. As we all know, the cell proliferation in cancers is very often considerably less than in normal tissues (*e.g.*, Ref. 6) indicating the probable occurrence of other major sites of control, such as cell loss, differentiation, etc.

The example of the stomach mentioned (their Ref. 3) documents that gastric cancer is often preceded by focal intestinal metaplasia and that the latter was seen to arise most often in the pyloric region

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The second point Dr. Stemmermann and colleagues make in their letter concerning carcinogens as inhibitors is not understood. How can one separate an “effect of the induction process” from a risk factor for induction? There is no clear evidence that cell proliferation that precedes an exposure to a carcinogen increases the risk of cancer. In fact, for some carcinogens, cell proliferation decreases the cytochrome P450 system and other enzymes, and these may decrease the risk for induction by decreasing the metabolic activation.

Their comment concerning radiation is not meaningful to me in the context of cell proliferation because we still have no solid data, only conjecture, about how radiation may induce cancer. Because cancer induced initially by radiation often takes a very long time, it is impossible to identify all the various factors including cell proliferation that might be involved at one or more steps in the process.

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Drs. Ames and Gold (2) and Drs. Cohen and Ellwein (3) base their major theses on the theoretical supposition that many, if not all, cancers are the result of mutations that are frequently generated by repeated cell proliferation. They then search for examples in human and experimental evidence that support this conjecture.

I have used quite the opposite approach. I look at human and animal cancer, including experimental, for evidence that cell proliferation by itself can generate cancer. I have yet to find such an example. Also, I have read all the references used by Dr. Ames, by Dr. Cohen and colleagues, and by many others as well, and I have yet to find a system in which it has been *shown* clearly (not supposed) that cell proliferation does in fact have this effect. There are many examples of cancer being induced by exposure to one of a variety of chemical carcinogens, radiation, or viruses by themselves with no other factors needed. Cell proliferation by itself does not. If the speculation with regard to cell proliferation, mutation, and cancer were valid, then obviously repeated cell proliferation should lead to cancer without the necessary dependence on exposure to chemical mutagens, viruses, or radiation.

In their various publications in which this thesis is presented, and in their letter (2), Drs. Ames and Gold present no objective evidence or data in support of this suggestion. For example, in the cited article by Cunningham *et al.* (their Ref. 11), there are so many other changes in phenotype that are induced by methapyrilene that to single out cell proliferation is both quite unjustified and unscientific.

Neither their own publications nor any of those in their reference list present even a single example of repeated cell proliferation by itself leading to cancer. To rationalize this, they use another supposition or speculation, the presumed origin of cancer only from putative stem cells. Neither they nor the articles referenced present any data that cancers of epithelial cells arise from stem cells. The publication they mention about stem cells, that of Shaver-Walker *et al.* (their Ref. 14), makes the claim that mutations induced by ethyl nitrosourea appear in stem cells in the small intestine of the mouse and concludes, by inference, that cancer development occurs from such cells. Stem cells were not isolated cleanly and also were defined in a strange way—cells that divide infrequently. Their procedure for the separation of different types of intestinal cells was anything but clean and is open to severe criticism. Also, the possible relevance of their finding to

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cancer was all by inference because they did not study cancer induction in the small intestine with ethyl nitrosourea.

One of the long-held theses, that cancers can arise from any cell that has retained the capacity to undergo cell proliferation, has no evidence against it and remains viable. Also, the commonly seen genomic instability in cancers described again recently by Loeb (7), and the dilemma of which mutations might precede cancer appearance and which are the result of cancer (Prehn; Ref. 8) are ignored by Drs. Ames and Gold. Presumably, they don't consider it to be very relevant.

Point 5 of the Ames and Gold letter deserves some comment. The relationship between chronic cell division in stem cells and in other cells that are discarded is totally misunderstood by Drs. Ames and Gold. The colon, as well as the jejunum and ileum, is proliferating and shedding cells all the time. This is true of every tissue in the body showing continuous cell proliferation. Why do the colon and stomach show high cancer rates even though they are shedding cells all the time while the small intestine does not? The same is also true in the skin. The excuse regarding stem cells and cells lost is not very helpful in understanding the dynamics of the tissues, especially in relation to cancer.

In the letter from Drs. Cohen and Ellwein (3), the authors unfortunately confuse "major risk factor" with "need." The need for a certain response pattern in a biological process, such as cancer development from "normal" or control tissue, does not make that response pattern a major risk factor. Cell proliferation is not "a hazard, a danger, a peril, or a dangerous element," as a risk is defined in several American and British English dictionaries. An example of cell proliferation being sufficient by itself to induce cancer or even to initiate or promote cancer has yet to be *shown*, instead of just hypothesized.

In their emphasis on "number of cell divisions" rather than the proliferation of cells with DNA synthesis (paragraph 2), the point Drs. Cohen and Ellwein attempt to make is not clear. In the many discussions in the literature of cell proliferation as a key risk factor for cancer, the focus is almost always on DNA replication. Increasing episodes of DNA replication lead to increasing numbers of mutations with increasing risk of cancer; that is the supposition. However, how "the total number of cell divisions" differs from the number of cell proliferations a cell goes through is not comprehensible.

Unless Drs. Cohen and Ellwein are referring to unpublished work, the data in the literature concerning ploidy and liver cancer are anything but clear. It is largely speculated (Seglen, Schwartz, and coworkers; Ref. 9) that the diploid cell is the precursor. Also, they state boldly that certain liver mitogens such as lead nitrate increase liver cells that are tetraploid or of greater ploidy. Again, the published data are anything but clear. The only data I am aware of concerning ploidy and liver cancer development assume that diploid cells are the precursors for nodules, yet no data concerning nodule to cancer sequence are presented. To base any conclusions on such "soft" and unconvincing evidence is indeed a dangerous way to assess risk factors for carcinogenesis.

Their discussion of their "biological model of carcinogenesis" is indeed entirely speculative. Their reference to the work of Knudson and other epidemiologists fails to indicate that the epidemiological approach to a chronic process like cancer can at best only indicate *the minimum number of steps*, not the actual number. This has been emphasized by outstanding epidemiologists such as Armitage (10). The number of cellular steps in the retina during the development of retinoblastoma is unknown and may well be several.

The discussion of psoriasis is indeed biased. I have read several articles and books on cell proliferation and psoriasis and have yet to see presented data that show that the cells involved in the proliferation are only the superficial cells and not the basal cells. Also, what

evidence do the authors have that skin cancer arises exclusively from so-called stem cells?

The discussion of the breast in pregnancy is indeed strange. It emphasizes that cell proliferation by itself is *not* a valid risk factor unless one relates it to biology.

With respect to their study with 2-acetylaminofluorene, because "tumors" apparently arise in the liver at all doses, yet no increase in cell proliferation is seen, how does one arrive at cell proliferation as a risk factor?

Drs. Cohen and Ellwein keep referring to the supposed origin of cancer precursor cells from stem cells rather than from differentiated cells. This is a very common misconception in biology and pathology. There is not a single example that I am aware of where an epithelial cancer or even an epithelial cell precursor of a cancer has been shown to have arisen from a supposed stem cell. This is a very common assumption without a single concrete example. For example, in the liver, where possible cells of origin for cancer can be followed geographically early in the process, the early foci, as precursors for nodules and ultimately a few cancers, arise *randomly* from mature hepatocytes without any evidence for a specialized cell as precursor.

In Dr. Butterworth's interesting response (4), he overlooks one fundamental and critical element in any analysis of carcinogenesis. Cancer in most organs and tissues, if not in all, originates in rare isolated cells that have acquired a new phenotype very early in the process. For example, in the liver, about which we know a fair amount, the altered hepatocytes with a new phenotype that appear during initiation with many different potent genotoxic carcinogens occur with a frequency of 1 per 10^5 – 10^6 original hepatocytes. These isolated hepatocytes show cell proliferation early, whereas the vast majority (almost 100%) of surrounding hepatocytes often show inhibition of cell proliferation, even with a strong regenerative mitogenic stimulus. These few rare cells further show more prolonged cell proliferation during promotion, whereas the surrounding cells do not. The measurement of overall cell proliferation in the liver under these conditions would miss almost completely the active carcinogen, if one uses cell proliferation as a major index of carcinogenic potential. Most, if not all, "genotoxic" carcinogens might well prove to be negative.

The principles that Dr. Butterworth used (as listed in his letter; Ref. 4) omit entirely any reference to the different cell populations, often very small, as playing a crucial role in the development of cancer. The emphasis on the tissue or organ as a whole obscures some of the key principles that are fundamental to our scientific analysis of cancer and carcinogenesis.

Also, underlying Dr. Butterworth's principles are the supposition and speculation that rare cells with the new phenotype are the result of mutations. It is, of course, well documented that mammals and other multicellular organisms have hundreds of cells with quite different phenotypes that appear regularly during their development from the fertilized single cell. There is no evidence that each of these different cells are mutants! The same may well be true in carcinogenesis, given the many biochemical changes induced by carcinogenic agents.

If the first principle listed by Dr. Butterworth is valid, then any cell with repeated cell proliferation should be a potential cancer cell, and at least some should be so. Why don't we see cancer arising in tissues with vigorous cell proliferation without the need for some exogenous carcinogenic stimulus?

As to Dr. Butterworth's last point, the decreasing emphasis on environmental chemicals is a logical consequence of the increasing emphasis on cell proliferation that is a major thesis in my article. This is indeed a dangerous position that is quite unacceptable to anyone interested in the prevention of cancer.

If we in cancer research are to be useful to the practitioners in medicine and in regulation, we must base our recommendations on

well-established scientific principles and not on vague unproved generalizations and suppositions. I feel certain that Dr. Butterworth and the vast majority of our colleagues would subscribe to this position. A reconsideration of the current "principles" may be in order. The need to emphasize valid mechanisms is clear.

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